

Post-flight Microbial Analysis of Samples from the International Space Station Water Recovery System and Oxygen Generation System

The Regenerative Environmental Control and Life Support System (ECLSS) on the International Space Station (ISS) includes the the Water Recovery System (WRS) and the Oxygen Generation System (OGS). The WRS consists of a Urine Processor Assembly (UPA) and Water Processor Assembly (WPA). This report describes microbial characterization of wastewater and surface samples collected from the WRS and OGS subsystems, returned to KSC, JSC, and MSFC on consecutive shuttle flights (STS-129 and STS-130) in 2009-10. STS-129 returned two filters that contained fluid samples from the WPA Waste Tank Orbital Recovery Unit (ORU), one from the waste tank and the other from the ISS humidity condensate. Direct count by microscopic enumeration revealed 8.38×10^4 cells per mL in the humidity condensate sample, but none of those cells were recoverable on solid agar media. In contrast, 3.32×10^5 cells per mL were measured from a surface swab of the WRS waste tank, including viable bacteria and fungi recovered after ≤ 12 days of incubation on solid agar media. Based on rDNA sequencing and phenotypic characterization, a fungus recovered from the filter was determined to be *Lecythophora mutabilis*. The bacterial isolate was identified by rDNA sequence data to be *Methylobacterium radiotolerans*. Additional UPA subsystem samples were returned on STS-130 for analysis. Both liquid and solid samples were collected from the Russian urine container (EDV), Distillation Assembly (DA) and Recycle Filter Tank Assembly (RFTA) for post-flight analysis. The bacterium *Pseudomonas aeruginosa* and fungus *Chaetomium brasiliense* were isolated from the EDV samples. No viable bacteria or fungi were recovered from RFTA brine samples (N = 6), but multiple samples (N = 11) from the DA and RFTA were found to contain fungal and bacterial cells. Many recovered cells have been identified to genus by rDNA sequencing and carbon source utilization profiling (BiOLOG Gen III). The presence of viable bacteria and fungi from WRS and OGS subsystems demonstrates the need for continued monitoring of ECLSS during future ISS operations and investigation of advanced antimicrobial controls.

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ABSTRACT

The Water Recovery System (WRS) onboard the International Space Station (ISS) consists of a Urine Processor Assembly (UPA) and Water Processor Assembly (WPA). This report describes microbial characterization of wastewater and surface samples collected from multiple WRS and OGS subsystems, returned to KSC, JSC, and MSFC on consecutive shuttle flights (STS-129 and STS-130) in 2009-10. STS-129 returned two filters that contained fluid samples from the WPA Waste Tank Orbital Recovery Unit (ORU), one from the waste tank and the other from the ISS humidity condensate. Direct count by microscopic enumeration revealed 8.38×10^4 cells per mL in the humidity condensate sample, but none of those cells were recoverable on solid agar media. In contrast, 3.32×10^5 cells per mL were measured from a surface swab of the WRS waste tank, including viable bacteria and fungi recovered after ≤ 12 days of incubation on solid agar media. Based on rDNA sequencing and phenotypic characterization, a fungus recovered from the filter was determined to be *Lecythophora mutabilis*. The bacterial isolate was identified by rDNA sequence data to be *Methylobacterium radiotolerans*. Additional UPA subsystem samples were returned on STS-130 for analysis. Both liquid and solid samples were collected from the Russian urine container (EDV), Distillation Assembly (DA) and Recycle Filter Tank Assembly (RFTA) for post-flight analysis. The bacterium *Pseudomonas aeruginosa* and fungus *Chaetomium brasiliense* were isolated from the EDV samples. No viable bacteria or fungi were recovered from RFTA brine samples (N = 6), but multiple samples (N = 11) from the DA and RFTA were found to contain fungal and bacterial cells. Many recovered cells have been identified to genus by rDNA sequencing and carbon source utilization profiling (BiOLOG Gen III). The presence of viable bacteria and fungi from WRS and OGS subsystems demonstrates the need for advanced antimicrobial control and monitoring of ECLSS during future ISS operations.

INTRODUCTION

The International Space Station (ISS) Regenerative Environmental Control and Life

Support Systems (ECLSS) includes the Water Recovery System (WRS) and Oxygen Generation

System (OGS), housed within three racks since they were installed in November 2008. A detailed description of the WRS and OGS architecture, including all relevant subsystems can be found in a review written by Carter (2010). Crew urine is delivered to the Urine Processor Assembly (UPA), which produces a distillate that is delivered to the Water Processor Assembly (WPA) Waste Tank. Humidity condensate (from ISS Common Cabin Air Assembly) is also delivered to the WPA, which processes the urine distillate and humidity condensate to potable water. The potable water is delivered to the ISS potable bus, where it is used by the crew for drinking water and hygiene, as flush water in the crew urinal, and as feed to the OGS. The OGS electrolyzes the water to hydrogen (vented to space) and oxygen for the crew.

Crew urine is transferred to the Urine Processor Assembly either directly from the US Segment Waste Hygiene Compartment (WHC) or transferred from the Russian Segment in a urine storage container referred to as an EDV. As urine is collected from the crew, it is pretreated with chromium trioxide and sulfuric acid to reduce the pH and therefore insure microbial control during the UPA process while also minimizing the generation of ammonia from urea. Figure 2 provides a simplified overview of the UPA. In the UPA, the pretreated urine is recycled through a Distillation Assembly. Here water and other volatiles are evaporated at low pressure and subsequently condensed to form the urine distillate that is delivered to the WPA waste tank. This distillation process generates a brine in the UPA recycle loop, which is periodically refurbished by replacing 44 L of volume via the Recycle Filter Tank Assembly (RFTA).

The WPA uses a series of treatment processes to remove particulates, dissolved organic and inorganic contaminants, and microbial contaminants. Figure 2 provides a simplified overview of the WPA. Waste water (urine distillate and humidity condensate) is collected in the Waste Tank, and subsequently fed to the Mostly Liquid Separator to remove free gas. The water is then pumped through the Particulate Filter and Multifiltration Beds to remove the inorganic contaminants and the majority of organic contaminants. Finally, the Catalytic Reactor removes the remaining organic contaminants (soluble organics not readily removed by adsorbents in the Multifiltration Beds) and bacterial/fungal species via thermal disinfection. Subsequent to the Catalytic Reactor, Ion Exchange removes any residual oxidation by-products, and residual iodine is added to the system as a biocide.

If left unchecked, biological growth can jeopardize missions by endangering crew health and causing hardware failures. While the WRS and OGS have demonstrated their safety and utility – already producing thousands of liters of potable water on ISS – several issues related to microbial growth have surfaced in just two years of operations. The purpose of this paper is to (1) identify which specific WRS systems continue to be challenged by microbial growth and (2) characterize the microbial composition (i.e., abundance and diversity) within these systems. By improving our understanding of where microbes continue to be problematic in the WRS, we can develop more effective countermeasures, ultimately making human spaceflight safer and more efficient.

The first microbe-related failure of the WRS occurred in June 2009 in the WPA in the Pump/Sep ORU, in a solenoid valve located between the WPA Waste Tank and the MLS.

Biomass from the Waste Tank had accumulated in passages in the solenoid valve, restricting flow to the point that the system could no longer function. An analysis of the pressure drop in the system indicated the restriction was in the Pump/Sep ORU, which was corroborated by the fact that the tightest restriction were in the solenoid valve. After replacing the ORU, the WPA returned to nominal operation. Subsequently, a filter was delivered for installation between the Waste Tank and the Pump/Sep ORU to protect the clearances in the solenoid valve. Upon ground inspection, biofouling was observed in the MLS inlet solenoid valve, blocking the flow of water in 11 out of 12 channels (Carter 2009). This study will report the variety of microbes recovered from the Pump/Sep ORU, in addition to samples from the humidity condensate and WPA Waste Tank feeding the MLS.

Another microbial incident took place in October 2009 in the UPA at the Distillation Assembly (DA). While the failure was not due to biomass, the precipitation happened because of a chemical measure designed to reduce microbial populations. The root cause of the DA malfunction was the buildup of calcium sulfate precipitates, which ultimately clogged the DA's pitot tube and caused the DA to flood. . Soluble calcium in astronaut urine (due to bone degradation in the reduced gravity of space) precipitated when mixed with the H_2SO_4 from the UPA water pretreatment. NASA is investigating options for removing calcium or inhibiting the precipitation of calcium sulfate. Understanding the microbial content of surrounding UPA subsystems, in the meantime, will help guide the DA redesign. Our study reports microbial analyses from the DA itself, shuttle and EDV urine containers, and the RFTA which accumulates and stores brine for disposal.

We also include samples here from the OGS whose primary function in ECLSS is oxygen generation. During OGS reactions, water is recirculated through an electrolysis process to form hydrogen and oxygen. The water recirculation loop chemistry has become an issue because of the unique environment. For reasons similar to the WRS, biofouling in the OGS can decrease overall system efficiency. In 2009, fluorescent and non-fluorescent particles were observed to be blocking the OGS filter screen. The root cause was determined to be a materials degradation issue rather than a microbial issue, but we assayed particle samples and included the results in this report.

MATERIALS & METHODS

1. Kennedy Space Center

The Applied Genetics and Technology Core labs (Dynamac Corp.) at the Kennedy Space Center (KSC) received various WRS and OGS samples collected on orbit during 2009-2010 ISS operations, spanning flights STS-129 and STS-130 (ISS Expeditions 19 thru 22). A description of each sample origin can be found in Tables 1-3. In summary, post-flight analyses included liquid, solid, and filtered samples from the following subsystems: (1) UPA: EDV, shuttle urine container, DA and RFTA; (2) WPA: humidity condensate tank, wastewater tank, Pump/Sep ORU, and Mostly Liquid Separator (MLS); and (3) OGS: surface swabs, water samples, and particulates.

Our microbial methods had two primary objectives: first, to generate plate count and direct count enumeration; second, to identify recovered bacterial and fungal isolates by rDNA sequencing and phenotypic characterization. Testing on each sample was conducted in

triplicate at a minimum. In some cases, samples required serial dilution for enumeration. The results were averaged and the standard deviation was calculated for both the Heterotrophic Plate Counts (HPC) and for the Acridine Orange Direct Counts (AODC). The AODC samples were sonicated, stained with 0.1% Acridine Orange (AO), and filtered onto 0.2µm 25mm black polycarbonate filters for enumeration on a Carl Zeiss Axioskop 2 epifluorescent microscope under oil immersion at 1000x magnification (Bloem 1995, Hobbie et al. 1977). The HPC test provides an estimate of the total number of bacteria in a sample that will develop into colonies during a period of incubation at a targeted temperature on a nutrient-rich agar. This test detects a broad group of bacteria including non-pathogens, pathogens, and opportunistic pathogens, but often does not accurately sample all of the bacteria in the sample examined.

Depending on whether samples were liquid, solid, or on filters, recovery methods varied slightly. For filter samples, a ¼ section of each filter was aseptically excised using a sterile scalpel and placed in 10 mL sterile Phosphate Buffered Saline (PBS)(Sigma). Microbiological characterization included light microscopy and plating of liquid samples on selective and non-selective growth media (R2A Agar, Plate Count Agar (PCA), Tryptic Soy Agar (TSA), Inhibitory Mold Agar (IMA), Malt Extract Agar (MEA), and Potato Dextrose Agar (PDA))(BD, BBL Difco) to recover viable bacteria and fungi. Solid and liquid WRS and OGS samples were processed directly onto selective and non-selective growth media. Plates were incubated at 30°C for fungi and 37°C for bacteria for up to 28 days. Fungal and bacterial isolates were aseptically streaked onto new agar plates for isolation and identification in the AGTC by rDNA sequencing (MicroSeq database, Applied Biosystems Inc.) and by carbon source utilization (Filamentous Fungi and GenIII bacterial databases, BIOLOG, Inc.).

The MicroSeq[®] D2 LSU rDNA Fungal Sequencing and the MicroSeq[®] 500 16s rDNA Bacterial Sequencing identification kits (ABI) were used to generate species-level identification for microbes isolated from the urine and fungal samples following the manufacturer's recommended protocol. DNA was isolated from cultivated microbes using the PrepMan™ Ultra Sample Preparation Reagent (ABI) and diluted 1:100 in molecular-grade water. The PCR Module from the kit used approximately 25 ng of genomic DNA on the Bio-Rad C1000 thermocycler. The PCR thermocycling conditions were: 95°C for 10 minutes, 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 45 seconds, and finished with 72°C for 10 minutes. PCR product was run on a SYBR Safe (Invitrogen) 2% agarose gel (Sigma) with the Benchtop pGEM[®] DNA markers (Promega) and visualized for quality and size. 5 µL of the PCR product was then purified with 2 µL of ExoSAP-IT[®] (USB) in duplicate. The 7µL of purified sample was then processed through the sequencing module of the kit. The cycle sequencing thermocycling conditions were: 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. The excess dye terminators and primers were removed the cycle sequencing reaction with the DyeEx 2.0 Spin Kit (Qiagen). 7.5 µL of Hi-Di™ Formamide (ABI) was loaded with 7.5 µL of the purified product on the ABI 3130 Genetic Analyzer. Most samples were analyzed for ATP production rates, inorganic chemistry content, and terminal restriction fragment length polymorphisms (T-RFLP); however, those data sets are not included.

2. Johnson Space Center

Characterization of the Pump/Sep ORU (from TT&E) and the OGS

Wastewater processing

RESULTS

1. Urine Processor Assembly

Samples from the UPA included US (N = 15) and Russian (N = 3) urine waste containers, solids from the DA (N = 1), and filters from the RFTA (N = 21). All urine waste container samples (N = 18) contained microorganisms (Table 4). Microscopic enumeration by AODC (which provided a total biomass quantity, with no distinction between living and dead microbes) revealed a range of 2.61×10^5 cells per mL to 8.05×10^8 cells per mL. The AODC range was similar comparing Russian and US samples. However, enumeration from heterotrophic plate counts (HPC) showed a difference. While US urine container samples were often too numerous to count by HPC, the number of cfu/mL from Russian containers was at the lower end of the detection limit for the assay. Based on rDNA sequencing and phenotypic characterization, the fungus *Chaetomium brasiliense* and bacteria *Pseudomonas aeruginosa* and *Staphylococcus hominis* were isolated from the EDV samples. Numerous species of bacteria and fungi were identified from US urine containers and are listed in Table 5 along with other UPA data.

While the only DA sample contained 2.69×10^7 cells per mL, none of the microbes were recoverable on media. In contrast, multiple RFTA sources (11 out of 18) yielded positive growth of microorganisms. Two RFTA samples had enumeration values too numerous to count, but the other nine were on the lower end of the HPC detection limit. Overall biomass values from AODC were variable, ranging from 8.10×10^4 cells per mL to 6.38×10^7 cells per mL. Fungi appeared more frequently in RFTA samples, including *Paecilomyces lilacinus*, *Aspergillus aculeatus*,

Lecythophora mutabilis, *Rhodotorula mucilaginosa* and *Geotrichum terrestre*. Bacteria identified from RFTA filters were *Alkalibacillus haloalkaliphilus* and *Lactococcus garvieae*.

2. Water Processor Assembly

Microbial data from the WPA included the Humidity Condensate Tank (N = 2), Wastewater Tank (N = 1), Separator ORU (N = 2), and MLS (Pump/Sep) (N = 14), arriving at KSC on the return of STS-130 as water samples, swabs, or filtrate. Microscopic enumeration of the liquid PBS wash by Acridine Orange Direct Count revealed high cell counts per mL across all WPA subsystems (consistent with UPA values), but viable bacteria or fungi were recovered primarily in the MLS samples and not the tanks or Separator ORU. After 12 days of incubation, growth occurred in only 2 out of 21 Humidity Condensate Tank selective media. Listed in Table 6, the fungi recovered were determined to be a single species identified as *Lecythophora mutabilis*, while the bacteria were *Cupriavidus metallidurans* and *Bacillus thuringiensis*. In the Wastewater Tank Filtrate, recovery of viable cells was similarly low, in just 3 out of 18 media plates (with only 1 to 2 microbial colonies per plate). *Methylobacterium radiotolerans* was the bacterium isolated and the fungus *Lecythophora mutabilis* was also present.

Microbial content from the Separator ORU was markedly different from MLS water samples. Viable PHC counts for the Separator ORU were low, but the diversity of cells isolated was high, including *Cupriavidus metallidurans* and *Bacillus thuringiensis* bacteria and *Lecythophora mutabilis* and *Cryptococcus curvatus* fungi. In contrast, the MLS subsystem had HPC numbers too high to count for 6 out of 14 water samples, but most of the isolates were

similar, including *Cupriavidus basilensis*, *Microbacterium laevaniformans*, and *Lecythophora mutabilis*. *Paecilomyces lilacinus* and *Candida incommunis* were also recovered.

3. Oxygen Generation System

Samples from the OGS originated from swabs, water samples, and particulates removed from the flight hardware after Expedition 19. Enumeration from AODC microscopy found that the biomass values were similar to UPA and WPA data. The same bacterial strain, *Rhodotorula mucilaginosa*, was isolated in 4 out of 6 samples after the growth period on selective media (Table 7). Based on HPC data for samples taken with moistened swabs, concentrations of the bacterium were very high, suggesting it the wet swab was a more effective method of removing microbes from the OGS. The samples with no viable microbes were from a sterile, unopened tube and a dry surface swab.

CONCLUSIONS

Every flight system assayed in this study contained a high amount of bacterial and fungal biomass, with many hosting viable, healthy cell populations. Our results demonstrate why biofouling can easily develop onboard ISS in the WRS and OGS. Altogether, 18 different species of bacteria and 14 different species of fungi were isolated from WPA, UPA, and OGS samples. Microbial identifications from this study are comparable to potable water, air, and on surfaces inside the ISS by Novikova et al. (2006). *Pseudomonas* sp., *Staphylococcus* sp., *Enterococcus* sp., *Proteus* sp., *Lactococcus* sp., *Moraxella* sp., *Bacillus* sp., and *Methylobacterium* sp. are genera found in our samples and also reported by Novikova et al. (2006). Fungal genera shared between the studies include *Chaetomium* sp., *Penicillium* sp., *Paecilomyces* sp., *Rhodotorula*

sp., *Candida* sp., *Cladosporium* sp., *Geotrichum* sp., and *Cryptococcus* sp. However, all other microbes we identified have not been previously reported in ISS or other spacecraft water-related samples. Implications from this finding are threefold: (1) our knowledge of the total microbial content onboard ISS is incomplete; (2) the effectiveness of antimicrobial treatments in ISS water processing systems can be improved; and (3) additional subsystem samples are needed, with a special emphasis on culture-independent microbiological assays.

While microbial species varied from system to system, several patterns emerged from our data. Shuttle urine containers were richest in microbial abundance and diversity, something that was expected since these samples were untreated with chemicals or other purification methods. The extent of viable bacteria and fungi within the RFTA samples was a surprising result, however. Water fed into the UPA is pretreated with chromium trioxide and H_2SO_4 to kill microbes, yet numerous viable cells from the RFTA demonstrate that the chemical pretreatment is ineffective or contamination has occurred downstream of the UPA inlet. Species in the RFTA – *Lactococcus garvieae*, *Aspergillus aculeatus* and *Geotrichum terrestre* – are also found in the urine container samples and may be resistant to H_2SO_4 treatments, passing through to the DA/RFTA cycling stage as viable cells. It would take just a few surviving microbes to quickly reestablish populations. Alternatively, these species may have been embedded in RFTA hardware prior to UPA operations began on ISS. In either case, because the microbes in the UPA are both plentiful and healthy, it helps explain the source of the Pump/Sep ORU failure in June 2009 where accumulated biomass clogged the MLS solenoid valve. As expected, most of the species we identified in our MLS Pump/Sep samples were also found in the RFTA.

The detection of bacteria and fungi in the WRS is not a new development, but it remains extremely relevant to future ISS operations. With construction almost complete, the space station can now house six permanent residents, all of whom contribute to the microbial contamination onboard. In the past, NASA has sanitized microbe-laced water from the WPA with iodine prior to crew consumption, and to date, no illnesses have been reported despite the presence of some pathogenic species (e.g. *Pseudomonas aeruginosa*) (La Duc et al. 2003). Even if microbes do not lead to issues with crew health, the buildup of biomass (both living and dead) is going to be accelerated by six permanent crew members on ISS. We predict higher incidences of biofouling in ECLSS if no changes are made to water processing systems. The root of most biofouling and healthy microbial populations seems to be the use of H_2SO_4 in the UPA. Sulfuric acid is not killing all microbes in wastewater pretreatments. Additionally, it can lead to the precipitation of calcium sulfate in the DA which has already caused a system failure. The current solution to accommodate H_2SO_4 issues is to periodically install filters upstream of the MLS inlet to keep water flowing through the WPA. By using a different chemical pretreatment, we multiple problems could be solved simultaneously. Future research should investigate alternatives to H_2SO_4 that are (1) compatible with the WRS and OGS subsystems and (2) can kill a broader range of microorganisms in UPA wastewater pretreatments.

Future investigations must also utilize culture-independent assays for characterizing microbial communities. Numerous bacteria and fungi have been listed in this study and a previous report by Novikova et al. (2006), yet both surveys used culture-based recovery methods for identifying microbes. Prior to rDNA sequencing, we first grew isolates on media which permitted the growth of only a fraction of the total microbial community present. In

reality, we expect the actual microbial content to be higher in abundance and diversity than what we have reported herein. For instance, microbes described by La Duc et al (2003) using culture independent methods were entirely different from our results, though both studies obtained samples from ISS water systems. State-of-the art assays, including microarrays and various metagenomic technologies should be prioritized by NASA for ensuring long-term operations of the WRS and OGS. Samples should be obtained from as many ECLSS locations onboard ISS as possible, in a systematic, standardized method so that temporal and spatial correlations are enabled. By getting more water samples and using better tools to monitor the microbial content in water processing systems, we can ensure safe and efficient operations on ISS far into the future.

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Table 1: Urine Processor Assembly Samples

Tracking #	Sample Origin	Sample Description	pH
1	Russian urine container (EDV)	EDV-U (1 of 2), pre-treated urine	4.42
2	Russian urine container (EDV)	EDV-U (2 of 2), pre-treated urine	1.85
13	Russian urine container (EDV)	EDV-Y 897	2.08
5	Shuttle urine container	STS-129 (2 of 2)	8.93
6	Shuttle urine container	STS-129 (1 of 2)	9.33
49	Shuttle urine container	STS-133, FCP-4 post-flight	7.10
50	Shuttle urine container	STS-133, FCP-3 post-flight (1 of 2)	8.76
51	Shuttle urine container	STS-133, FCP-3 post-flight (2 of 2)	8.78
52	Shuttle urine container	STS-135, pre-flight (1 of 2)	7.19
53	Shuttle urine container	STS-135, pre-flight (2 of 2)	7.41
58	Shuttle urine container	STS-133, FCP3	9.24
59	Shuttle urine container	STS-134, WasteTank	3.33
60	Shuttle urine container	STS-133, FCP-7	6.37
61	Shuttle urine container	STS-132, GN04	7.15
62	Shuttle urine container	STS-131, Wastewater bag A	7.30
63	Shuttle urine container	STS-131, Urine distillate bag A	7.14
64	Shuttle urine container	STS-133, FCP-4 drain of dump nozzle	7.91
65	Shuttle urine container	STS-132, ULF-4 wastewater	7.14
7	Distillation Assembly (DA)	STS-129, S/N002	4.59
3	Recycle Filter Tank Assembly (RFTA)	S/N006 (1 of 2), brine	3.07
4	Recycle Filter Tank Assembly (RFTA)	S/N006 (2 of 2), brine	1.57
14	Recycle Filter Tank Assembly (RFTA)	S/N001 (1 of 2)	1.68
15	Recycle Filter Tank Assembly (RFTA)	S/N001 (2 of 2)	1.82
16	Recycle Filter Tank Assembly (RFTA)	S/N005 (1 of 2)	1.46
17	Recycle Filter Tank Assembly (RFTA)	S/N005 (2 of 2)	1.47
18	Recycle Filter Tank Assembly (RFTA)	S/N001, F1 filter (1 of 2)	N/A
19	Recycle Filter Tank Assembly (RFTA)	S/N001, F4 backflow	N/A
20	Recycle Filter Tank Assembly (RFTA)	S/N001, F4 filtrate	N/A
21	Recycle Filter Tank Assembly (RFTA)	S/N001, F1 filter (2 of 2)	N/A
22	Recycle Filter Tank Assembly (RFTA)	S/N001, F4 filter	N/A
23	Recycle Filter Tank Assembly (RFTA)	S/N005, F4 filter	N/A
24	Recycle Filter Tank Assembly (RFTA)	S/N005, F1 purge pump	N/A
25	Recycle Filter Tank Assembly (RFTA)	S/N005, F4 filtrate	N/A
26	Recycle Filter Tank Assembly (RFTA)	S/N005, F4 backflow	N/A
27	Recycle Filter Tank Assembly (RFTA)	S/N005, F1 directional	N/A
28	Recycle Filter Tank Assembly (RFTA)	S/N005, F1 backflow	N/A
54	Recycle Filter Tank Assembly (RFTA)	SN007 (1 of 2)	1.81
55	Recycle Filter Tank Assembly (RFTA)	SN007 (2 of 2)	1.80
56	Recycle Filter Tank Assembly (RFTA)	SN008 (1 of 2)	1.65
57	Recycle Filter Tank Assembly (RFTA)	SN008 (2 of 2)	1.60

Table 2: Water Processor Assembly Samples

Tracking #	Sample Origin	Sample Description	pH
8	Humidity Condensate Tank	20 mL filtrate	N/A
10	Humidity Condensate Tank	STS-130A	7.73
9	Wastewater Tank	50 mL filtrate	N/A
11	Separator ORU	STS-130A	7.51
12	Separator ORU	STS-130A	5.54
29	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, OCT6T to pump inlet hose	N/A
32	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Seat swab	N/A
33	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Seat swab page hole	N/A
34	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Seat swab stem	N/A
31	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water from outlet tube	N/A
30	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water (1 of 9)	N/A
41	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water (2 of 9)	N/A
42	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water (3 of 9)	N/A
43	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water (4 of 9)	N/A
44	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water (5 of 9)	N/A
45	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water (6 of 9)	N/A
46	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water (7 of 9)	N/A
47	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water (8 of 9)	N/A
48	Mostly Liquid Separator (MLS)	Pump/Sep S/N001, Water (9 of 9)	N/A

Table 3: Oxygen Generator System Samples

Tracking #	Sample Origin	Sample Description	pH
35	Empty unopened sterile tube	ISS-19A	N/A
36	Amresco water	ISS-19A	N/A
37	Swab moistened with water	ISS-19A	N/A
38	Dry swab w/ non-fluorescent particles	ISS-19A	N/A
39	Moistened swab w/ non-fluorescent particles	ISS-19A	N/A
40	Moistened swab w/ fluorescent particles	ISS-19A	N/A

Table 4: Enumeration Data

Tracking #	AO (cells/mL)	HPC (cfu/mL)	Tracking #	AO (cells/mL)	HPC (cfu/mL)
UPA					
1	5.18E+07	5.26E-02	25	4.84E+05	TNTC
2	5.52E+07	5.26E-02	27	8.10E+04	0.00E+00
13	3.31E+06	1.50E+00	54	2.98E+07	2.80E+02
5	7.22E+07	1.62E+03	55	2.77E+07	0.00E+00
6	7.33E+07	1.44E+03	56	1.28E+07	0.00E+00
49	8.05E+08	TNTC	57	1.52E+07	0.00E+00
50	1.85E+08	TNTC	WPA		
51	1.87E+08	TNTC	8	9.29E+04	0.00E+00
52	1.16E+08	2.90E+02	10	7.67E+06	2.00E+01
53	1.16E+08	6.90E+02	9	6.67E+05	2.78E-01
58	1.04E+08	5.34E+05	11	5.22E+06	4.50E+00
59	2.61E+05	2.70E+03	12	7.86E+05	0.00E+00
60	2.45E+07	5.00E+02	29	4.21E+07	TNTC
61	3.67E+07	6.30E+03	32	5.39E+07	TNTC
62	4.22E+07	2.19E+05	33	1.04E+07	TNTC
63	1.47E+06	7.25E+05	34	1.73E+07	TNTC
64	3.51E+08	2.72E+05	31	2.96E+07	TNTC
65	1.02E+07	TNTC	30	2.96E+07	TNTC
7	2.69E+07	0.00E+00	OGS		
3	6.38E+07	0.00E+00	35	6.11E+05	0.00E+00
4	4.53E+07	0.00E+00	36	2.98E+05	1.00E+01
14	2.40E+07	0.00E+00	37	4.28E+05	1.00E+01
15	2.67E+07	0.00E+00	38	1.94E+06	0.00E+00
16	2.97E+07	0.00E+00	39	1.14E+06	TNTC
17	3.60E+07	0.00E+00	40	5.72E+05	TNTC
18	4.63E+05	0.00E+00			
20	3.03E+05	6.68E+00			
21	1.23E+05	7.34E+00			
22	1.01E+06	8.33E+00			
23	6.11E+05	TNTC			
24	1.10E+05	0.00E+00			

Table 5: Urine Processor Assembly Microbial Identification

Tracking #	Bacterial Identification	Fungal Identification
1		<i>Chaetomium brasiliense</i>
2	<i>Pseudomonas aeruginosa</i>	
13	<i>Staphylococcus hominis</i>	
5	<i>Enterococcus faecalis</i> , <i>Eubacterium contortum</i>	
6	<i>Clostridium sporosphaeroides</i> , <i>Tissierella praeacuta</i> , <i>Enterococcus faecalis</i>	<i>Penicillium</i> sp.
49	<i>Citrobacter koseri</i> , <i>Lyssinibacillus fusiformis</i> , <i>Proteus mirabilis</i>	<i>Paecilomyces lilacinus</i>
50	<i>Enterococcus gallinarum</i> , <i>Lactococcus garvieae</i>	
51	<i>Lyssinibacillus fusiformis</i> , <i>Enterococcus gallinarum</i> , <i>L. garvieae</i>	
52	<i>Citrobacter koseri</i> , <i>Pseudomonas veronii</i> , <i>Lactococcus garvieae</i>	<i>Debaryomyces hansenii</i>
53	<i>Citrobacter koseri</i> , <i>Cupriavidus metallidurans</i> , <i>Lactococcus garvieae</i>	<i>Debaryomyces hansenii</i>
58	<i>Burkholderia cepacia</i> , <i>Moraxella</i> sp.	<i>Lecythophora mutabilis</i> , <i>Rhodotorula mucilaginosa</i>
59		<i>Bulleromyces albus</i> , <i>Candida</i> sp., <i>Lecythophora mutabilis</i> , <i>Rhodotorula mucilaginosa</i> , <i>Wingea robertsiae</i>
60	<i>Burkholderia cepacia</i> , <i>Citrobacter koseri</i> , <i>Moraxella</i> sp.	<i>Candida sake</i> , <i>Wingea robertsiae</i>
61	<i>Burkholderia cepacia</i>	<i>Candida sake</i> , <i>Wingea robertsiae</i> , <i>Bulleromyces albus</i>
62	<i>Burkholderia cepacia</i> , <i>Moraxella</i> sp.	<i>Lecythophora mutabilis</i> , <i>Candida sake</i> , <i>Bulleromyces albus</i> , <i>Wingea robertsiae</i>
63	<i>Citrobacter koseri</i> , <i>Burkholderia cepacia</i>	<i>Lecythophora mutabilis</i> , <i>Candida</i> sp., <i>Cladosporium</i> sp., <i>Wingea robertsiae</i>
64	<i>Citrobacter koseri</i> , <i>Burkholderia cepacia</i> , <i>Moraxella</i> sp.	
65	<i>Burkholderia cepacia</i> , <i>Moraxella</i> sp.	<i>Lecythophora mutabilis</i> , <i>Pichia guilliermondii</i>
20		<i>Paecilomyces lilacinus</i>
21		<i>Aspergillus aculeatus</i>
22	<i>Alkalibacillus haloalkaliphilus</i>	<i>Aspergillus aculeatus</i> , <i>Paecilomyces lilacinus</i>
23		<i>Lecythophora mutabilis</i>
25		<i>Paecilomyces lilacinus</i>
26		<i>Paecilomyces lilacinus</i>
54	<i>Lactococcus garvieae</i>	
55		<i>Rhodotorula mucilaginosa</i> , <i>Geotrichum terrestre</i>
56		<i>Rhodotorula mucilaginosa</i> , <i>Geotrichum terrestre</i>
57		<i>Rhodotorula mucilaginosa</i> , <i>Geotrichum terrestre</i>

Table 6: Water Processor Assembly Microbial Identification

Tracking #	Bacterial Identification	Fungal Identification
10	<i>Cupriavidus metallidurans, Bacillus thuringiensis</i>	<i>Lecythophora mutabilis</i>
9	<i>Methylobacterium radiotolerans</i>	<i>Lecythophora mutabilis</i>
11	<i>Cupriavidus metallidurans, Bacillus thuringiensis</i>	<i>Lecythophora mutabilis</i>
12	<i>Bacillus thuringiensis, Cupriavidus metallidurans</i>	<i>Cryptococcus curvatus</i>
29	<i>Cupriavidus basilensis</i>	<i>Lecythophora mutabilis, Paecilomyces lilacinus</i>
32	<i>Cupriavidus basilensis, Microbacterium laeviformans</i>	<i>Candida incommunis</i>
33	<i>Cupriavidus basilensis, Microbacterium laeviformans</i>	
34	<i>Cupriavidus basilensis, Microbacterium laeviformans</i>	
31	<i>Cupriavidus basilensis</i>	<i>Lecythophora mutabilis</i>
30	<i>Cupriavidus basilensis, Microbacterium laeviformans</i>	<i>Lecythophora mutabilis, Paecilomyces lilacinus</i>
41		<i>Lecythophora mutabilis</i>
42		<i>Lecythophora mutabilis</i>
43		<i>Lecythophora mutabilis</i>
44		<i>Lecythophora mutabilis</i>
45		<i>Lecythophora mutabilis</i>
46		<i>Paecilomyces lilacinus</i>
47		<i>Paecilomyces lilacinus</i>
48		<i>Paecilomyces lilacinus</i>

Table 7: Oxygen Generator System Microbial Identification

Tracking #	Bacterial Identification	Fungal Identification
36		<i>Rhodotorula mucilaginosa</i>
37		<i>Rhodotorula mucilaginosa</i>
39		<i>Rhodotorula mucilaginosa</i>
40		<i>Rhodotorula mucilaginosa</i>